

What is claimed is:

1. A vaccine for Newcastle disease comprising a Newcastle disease virus Z, wherein the Newcastle disease virus Z has at least two of the features selected from the group consisting of (1) a  $F_0$  protein cleavage site having at least two less basic amino acid residues than a  $F_0$  protein cleavage site of Newcastle disease virus wild type strain Beaudette C; (2) an amino acid having a non-aromatic side chain at the N terminus of the  $F_1$  cleavage fragment, wherein the amino acid having a non-aromatic side chain is glycine, alanine, valine, leucine or isoleucine; and (3) an open reading frame of a HN glycoprotein being longer than an open reading frame of a HN glycoprotein of Newcastle disease virus wild type strain Beaudette C.
2. The vaccine of claim 1, wherein in (1) the Newcastle disease virus Z has the  $F_0$  protein cleavage site having serine or glycine independently replacing at least two basic amino acid residues of the  $F_0$  protein cleavage site of Newcastle disease virus wild type strain Beaudette C.
3. The vaccine of claim 2, wherein in (1) said at least two basic amino acid residues of the  $F_0$  protein cleavage site of Newcastle disease virus wild type strain Beaudette C replaced by serine or glycine are arginine or lysine.
4. The vaccine of claim 3, wherein in (1) the Newcastle disease virus Z has at least one of the following two features: (i) a codon, TCC, for serine in place of the codon for an arginine residue at the -2 position of the  $F_0$  protein cleavage site of Newcastle disease virus wild type strain Beaudette C, and (ii) a codon, TCC, for serine in place of the codon for an arginine residue at the -5 position of the  $F_0$  protein cleavage site of Newcastle disease virus wild type strain Beaudette C.
5. The vaccine of claim 1, wherein in (2) the amino acid having a non-aromatic side chain is leucine.
6. The vaccine of claim 4, wherein the Newcastle disease virus Z has at least one of the following two features: (i) a codon, TCC, for serine in place of the codon for an arginine residue at the -2 position of the  $F_0$  protein cleavage site of Newcastle disease virus wild type strain Beaudette C, and (ii) a codon, TCC, for serine in place of the codon for an arginine residue at the -5 position of the  $F_0$  protein

cleavage site of Newcastle disease virus wild type strain Beaudette C; and has an amino acid having a non-aromatic side chain at the N terminus of the F<sub>1</sub> cleavage fragment, wherein the amino acid having a non-aromatic side chain is leucine.

7. The vaccine of claim 6, wherein the codon for leucine is CTC.

5 8. An isolated nucleic acid comprising a sequence of 15,186 nucleotides as described in Figure 2.

9. An isolated nucleic acid of up to 200 nucleotides in length, comprising a sequence of 55 nucleotides of the leader region described in Figure 2.

10 10. An isolated nucleic acid of up to 350 nucleotides in length, comprising a sequence of 113 nucleotides of the trailer region described in Figure 2.

11. An isolated nucleic acid of up to 2500 nucleotides in length, comprising the nucleotide sequence of the NP region described in Figure 2, wherein the nucleotide sequence of the NP region is available from the GenBank database with the accession number AF064091.

15 12. The isolated nucleic acid of claim 11 consisting of the nucleotide sequence of the NP region.

13. A method of producing a Newcastle disease virus, said method comprising the following steps:

20 (1). providing a plasmid comprising a promoter and a cDNA encoding the antigenome of Newcastle disease virus;

(2). providing a plasmid comprising the gene for Newcastle disease virus NP protein under the control of a promoter;

(3). providing a plasmid comprising the gene for Newcastle disease virus P protein under the control of a promoter;

25 (4). providing a plasmid comprising the gene for Newcastle disease virus L protein under the control of a promoter;

(5). transfecting cells in a medium with a mixture of the plasmids of steps (1)-(4); and thereafter

(6). isolating the Newcastle disease virus from the cells or the medium.

30 14. The method of claim 13, wherein a leader end of the cDNA in step (1) is joined with a promoter for T7 RNA polymerase, the promoter in steps (2)-(4) are

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promoters for T7 RNA polymerase, and the cells in step 5 are also transfected with vaccinia virus that expresses T7 RNA polymerase.

15        15.     The method of claim 13, wherein a leader end of the cDNA in step (1) is joined with a promoter for T7 RNA polymerase and a trailer end of the cDNA in step (1) is joined with hepatitis delta virus antigenome ribozyme sequence followed by tandem terminators of T7 transcription, the promoter in steps (2)-(4) are promoters for T7 RNA polymerase, and the cells in step 5 are also transfected with vaccinia virus that expresses T7 RNA polymerase.

10        16.     The method of claim 13, wherein the cDNA contains at least one, e.g. 2 or 3, restriction sites as markers.

17.     The method of claim 13, wherein the cells in step (5) are avian cells.

18.     The method of claim 17, wherein the avian cells are HEp-2 cells.

15        19.     The vaccine of claim 1, wherein in (1), the F<sub>0</sub> protein cleavage site has at least two less basic amino acid residues than a F<sub>0</sub> protein cleavage site of Newcastle disease virus wild type strain Beaudette C, wherein said at least two basic amino acid residues are arginine or lysine.

20.     An isolated protein encoded by the nucleic acid of claim 11.

21.     A synthetic cDNA which encodes an infectious Newcastle disease virus.

20        22.     A vector containing the cDNA of claim 21.

23.     A host cell containing the cDNA of claim 21.

24.     A method of producing infectious Newcastle disease virus, comprising the following steps:

25        inserting a cDNA of claim 21 into a host cell, wherein the cDNA is operably-linked to a promoter; and

expressing the cDNA in the host cell to product the infectious Newcastle disease virus.

25.     The method of claim 24, further comprising purifying the infectious Newcastle disease virus.

30        26.     The vaccine of claim 1, wherein the Newcastle disease virus Z carries at least one gene encoding an avian cytokine.

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